

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

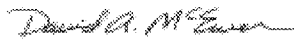
EPA MRID Number 48718011

**Data Requirement:**

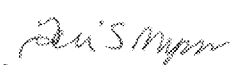
PMRA Data Code	{.....}
EPA DP Barcode	402518
OECD Data Point	{.....}
EPA MRID	48718011
EPA Guideline	850.1400/850.1500

**Test material:** Dicamba acid **Purity:** 93.9%  
**Common name:** Dicamba  
**Chemical name:** IUPAC: 3,6-dichloro-o-anisic acid  
CAS name: 3,6-dichloro-2-methoxybenzoic acid  
CAS No.: 1918-00-9  
Synonyms: BAS 183 H

**Primary Reviewer:** David A. McEwen  
**Staff Scientist, CSS-Dynamac Corporation**


**Signature:**   
**Date:** 12/05/12

**Secondary Reviewer:** Teri S. Myers  
**Environmental Scientist, CDM Smith**

**Signature:**   
**Date:** 01/25/13

**Primary Reviewer:** Elizabeth Donovan, Biologist  
**EPA/EFED/ERB 6**

**Date:** 9/7/2016

  
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**Secondary Reviewer(s):** Amy Blankinship, Senior Scientist  
**EPA/EFED/ERB 6**

**Date:** 11/2/2016

**Reference/Submission No.:** {.....}

<b>Company Code</b>	{.....}	[For PMRA]
<b>Active Code</b>	{.....}	[For PMRA]
<b>Use Site Category</b>	{.....}	[For PMRA]
<b>EPA PC Code</b>	029801	

**Date Evaluation Completed:** 11-2-2016

**CITATION:** Minderhout, T, T.Z. Kendall, and S.P. Gallagher. 2012. Dicamba Acid: An Early Life-Stage Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*). Unpublished study performed by Wildlife International Ltd., Easton, MD. Laboratory Study No. 147A-278B. Study sponsored by BASF Corporation, Research Triangle Park, NC. Study initiated August 18, 2011 and completed January 11, 2012.

**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the toxicity of a pesticide to fish, early life cycle. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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PMRA Submission Number {.....}

EPA MRID Number 48718011

## EXECUTIVE SUMMARY:

The 34-day chronic toxicity of dicamba acid to the early life-stage of sheepshead minnow (*Cyprinodon variegatus*) was studied under flow-through conditions. Fertilized sheepshead minnow embryos (80/level, <27 hours old) were exposed at nominal concentrations of 0 (negative and solvent controls), 0.31, 0.77, 1.9, 4.8, and 12 mg ai/L. Mean-measured concentrations were <0.100 (<LOQ, controls), 0.28, 0.72, 1.8, 4.5, and 11 mg ai/L, respectively. The test system was maintained at 23.6 to 25.8°C and a pH of 7.7 to 8.0. There were no treatment-related effects observed upon time to hatch, hatching success, post-hatch larval survival, or clinical signs of toxicity. Significant reductions relative to the negative control ( $p < 0.05$ ) were detected for length at the 0.28, 0.72, 4.5, and 11 mg ai/L levels; these inhibitions were consistent, ranging from 2-5% of negative control lengths over the concentration range which was approximately one magnitude order. Significant reductions relative to the negative control were also detected for wet and dry weight at the 0.28 and 4.5 mg ai/L levels; the magnitude of these reductions was greater (i.e., 9-13% lower than negative control weights) than that for length. The dose response for these endpoints, especially for length, was particularly flat over an order of magnitude in dosing. While slight reductions from negative control were observed, they do not appear to be dose-dependent.

This study is classified as scientifically sound and does satisfy guideline requirements for an early life stage toxicity study with fish.

## **Results Synopsis**

Test Organism Size/Age (mean Weight or Length): Embryos, <27 hours old

Test Type (Flow-through, Static, Static Renewal): Flow-through

NOAEC: 11mg ai/L

LOAEC: > 11 mg ai/L

Endpoint(s) affected: none

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

**I. MATERIALS AND METHODS**

**GUIDELINE(S) FOLLOWED:** This study was conducted following guidelines outlined in the U.S. EPA Ecological Effects Test Guideline 850.1400: *Fish Early-Life Stage Toxicity Test* (draft, 1996) and ASTM Standard E1241-05, *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish* (2005).

The following deviations from OCSPP 850.1400 guidance were observed:

- The embryos (<27 hours old) were slightly older than recommended (2 to 24 hours old).

This deviation does not affect the scientific soundness or acceptability of this study.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in accordance with GLP Standards as published by the U.S. EPA (40 CFR Parts 160 and 792), OECD Principles of GLP [ENV/MC/CHEM(98)17], and Japan MAFF (11 NohSan, Notification No. 6283, 1999), with the following exception: periodic analysis of salt water for potential contaminants. It was reported that the periodic analyses were performed using a certified laboratory and standard U.S. EPA analytical methods.

**A. MATERIALS:**

**1. Test Material** Dicamba acid

**Description:** Solid

**Lot No./Batch No. :** 0002B01BA-251

**Purity:** 93.9%

**Stability of compound under test conditions:** Stable, as determined from weekly analyses of test solutions at all treatment levels.

**Storage conditions of Test chemicals:** Under ambient conditions

**Physicochemical properties of dicamba acid.**

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

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**2. Test organism:**

<b>Species:</b>	Sheepshead minnow ( <i>Cyprinodon variegatus</i> ) [EPA recommends any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See Standard Evaluation Procedure for listing of recommended species. OECD recommends rainbow trout, fathead minnows, zebra fish, and ricefish but does not exclude the use of other species.]
<b>Age /embryonic stage at test initiation:</b>	Embryos, <27 hours post-fertilization. [EPA recommends fish embryos 2 to 24 hours old.]
<b>Method of collection of the fertilized eggs:</b>	Eggs were purchased and arrived free-floating. They were examined under a dissecting microscope to select healthy viable specimens at approximately the same stage of development.
<b>Source:</b>	Aquatic BioSystems, Inc., Fort Collins, CO

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a. Range-finding study: The concentrations were selected in consultation with the Sponsor and were based upon the results of preliminary exploratory range-finding toxicity data. A 19-day post-hatch preliminary study was conducted with 50 embryos per level (divided equally into two replicates) at nominal concentrations of 0 (negative control), 0 (0.1 mL DMF/L control), 0.081, 0.27, 0.90, 3.0, and 10 mg ai/L. No treatment-related effects were observed upon hatching success, post-hatch survival (assessed on Day 19), or wet weight at any exposure level (data provided).

b. Definitive study

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		Criteria
<u>Parental acclimation, if any</u> Period:  Conditions (same as test or not):  Feeding (type, source, amount given, frequency):  Health (any mortality observed):	N/A	Embryos used in the test were collected from at least 10 spawns from 3 male and 8 female adults.
Number of fertilized eggs/embryos in each treatment at test initiation:	80 embryos per treatment level, divided into 20 embryos per cup, one cup per replicate, and four replicate aquaria per level.	Alevins were not thinned.  <i>Each treatment should include a minimum of 20 embryos per replicate cup and a minimum of 30 fish per treatment for post-hatch exposure (OECD recommends at least 60 eggs, divided between at least 2 replicates)</i>
<u>Concentration of test material</u> Nominal:  Mean measured:	0 (negative and solvent controls), 0.31, 0.77, 1.9, 4.8, and 12 mg ai/L  <0.100 (<LOQ, controls), 0.28, 0.72, 1.8, 4.5, and 11 mg ai/L	Nominal concentrations were spaced by a factor of 2.5.  For concentration verification, samples were collected from alternating replicate chambers on Days 0, 7, 14, 21, 28, and 34.  Minimal analytical variation was observed for all levels, with recoveries ranging from 87.6 to 104% of nominal concentrations.  <i>A minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate should be used.</i> <i>- Toxicant concentration should be measured in one tank at each toxicant level every week.</i> <i>- One concentration should adversely affect a life stage and one concentration should not affect any life stage.</i> <i>OECD recommends that 5 concentrations be spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution should be within <math>\pm 20\%</math> of the mean measured values.</i>

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

Parameter	Details	Remarks
		Criteria
Solvent (type, percentage, if used):	Dimethyl formamide (DMF) 0.02 mL/L	<p>The solvent should not exceed 0.1 ml/L in a flow-through system. Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, and ethanol.</p> <p>OECD recommends that the solvent not have an effect on survival nor produce any other adverse effects; concentration should not be greater than 0.1 ml/L.</p>
<u>Number of replicates</u> Control: Solvent control: Treated ones:	4 4 4/level	<p>Number of replicates should be 4 per concentration.</p> <p>A solvent control should be used in conjunction with a solubilizing agent.</p>

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

Parameter	Details	Remarks
		<i>Criteria</i>
<u>Test condition</u>  Static renewal/flow-through:  Type of dilution system for flow through method:  Flow rate:  Renewal rate for static renewal:	Flow-through  Continuous-flow diluter  <i>ca.</i> 10 volume exchanges per day  N/A	<p>The syringe pumps and rotameters were calibrated prior to the test and the rotameters were verified weekly thereafter. The proportion of water that was split into each replicate chamber was also checked prior to the test and weekly during the test to ensure that flow rates varied by no more than <math>\pm 10\%</math> of the mean flow rate. The general operation of the diluter was visually checked generally twice daily.</p> <hr/> <p><i>Intermittent flow proportional diluters or continuous flow serial diluters should be used. EPA recommends that flow rate to larval cups should provide 90% replacement in 8 to 12 hours (OECD recommends 5 test chamber volumes/24 hours). For static-renewal, OECD recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or retain organisms in vessels and change at least 2/3 test water. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used.</i></p> <p><i>Toxicant Mixing:</i>  1) Mixing chamber is preferred;  2) Aeration should not be used for mixing;  3) The test solution should be completely mixed before introduction into the test system;  4) Flow splitting accuracy should be within 10%.</p>
Aeration, if any:	None reported	<hr/> <p><i>Dilution water should be aerated to ensure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.</i></p>
Duration of the test:	34 days: 6-day hatching period and 28-day post-hatch period	<p>Acceptable for this species under OCSPP guidance.</p> <hr/> <p><i>Recommended test duration is 32 days for EPA. OECD recommendations for test duration are species specific and range from 28-60 days.</i></p>

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

Parameter	Details	Remarks
		<i>Criteria</i>
<u>Embryo cups, if used</u> Type/material (glass/stainless steel):  Size:  Fill volume:	Glass cylinders with 425 µm nylon screen mesh bottoms attached with silicone adhesive  50 mm diameter  Not reported	One embryo cup was suspended in each replicate vessel and was oscillated at 2 rpm.  <i>Recommended embryo cups are 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</i>
<u>Test vessel</u> Type/material: (glass/stainless steel)  Size:  Fill volume:	Glass  9 L  7 L (ca. 15.8 cm depth)	<i>Recommended test vessel is all glass or glass with stainless steel frame.</i>
Source of dilution water	<p>Natural sea water was collected at Indian River Inlet, DE and sand-filtered to remove particles &gt;25 µm. The filtered water was diluted to a salinity of approximately 20‰ with fresh water from an on-site well, and then aerated. Prior to delivery to the diluter system, the dilution water was filtered to 0.45 µm and UV-sterilized.</p> <p>During the 4-week period immediately preceding the study, the salinity of the dilution water was 20‰ and the pH ranged from 8.0-8.1 (n=4).</p>	<p>Results of periodic analysis for pesticides, organics, and metals were provided from water collected on 12/29/10.</p> <p><i>Source of dilution water should be natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants. OECD accepts any water in which the test species show control survival at least as good as presented in SEP.</i></p>

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

Parameter	Details	Remarks
		<i>Criteria</i>
<u>Water parameters</u> Hardness: pH: Dissolved oxygen:  Temperature(s) (record all the temperatures used for different life stages):  Photoperiod:  Salinity (for marine or estuarine species): Other measurements:  Interval of water quality measurements:	Not determined 7.7 to 8.0 ≥65% saturation (≥4.8 mg/L)  Weekly: 23.6 to 25.8°C Continuous: 24 to 26°C  16 hours light/8 hours dark, with 30-minute transition periods  20‰ N/A  Temperature was measured in each chamber at least weekly and in one negative control replicate continuously. DO and pH were measured in alternating replicates from all levels at least weekly. Salinity was measured in one alternating replicate of the negative control and highest concentration level at least weekly.	Light intensity, measured at test initiation over the water surface of one representative chamber, was 789 lux.  <i>Recommended hardness: 40-48 mg/L as CaCO<sub>3</sub>;</i> <i>Recommended pH: 7.2 to 7.6</i> <i>Dissolved Oxygen (DO) should be measured at each concentration at least once a week;</i> <i>Freshwater parameters in a control and one concentration should be analyzed once a week.</i> <i>Temperature depends upon test species and should not deviate by more than 2°C from appropriate temperature.</i> <i>OECD recommends that DO concentration be between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test.</i> <i>Temperature should be measured continuously.</i>
<u>Post-hatch details</u> When the post-hatch period began:  Number of hatched eggs (alevins)/ treatment released to the test chamber:  On what day, the alevins were released from the incubation cups to the test chamber:	Day 6  All surviving alevins   Day 6	OCSPP specifies a control hatching success criterion of >75% and a post-hatch survival of 80%. Both criteria were satisfied.  Any unhatched embryos were kept in the egg cup until they hatched and were released into the test chamber (or until death of the embryo occurred).  <i>Percentage of embryos that produce live fry should be ≥ 50% in each control; percentage of hatch in any control embryo cup should not be more than 1.6 times that in another control cup.</i>

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

Parameter	Details	Remarks
		Criteria
<u>Post-hatch Feeding</u> Start date:  Type/source of feed:  Amount given:  Frequency of feeding:	Day 6  Live brine shrimp ( <i>Artemia sp.</i> ) nauplii (Brine Shrimp Direct, Ogden, UT)  Not reported; however it was noted that rations were adjusted each week to account for losses due to mortality  Two to three times per day	The fish were not fed for at least 48 hours prior to test termination.
Recovery of chemical:  Frequency of measurement:  LOD: LOQ:	87.6 to 104%  At least once weekly  0.000119 mg ai/L 0.100 mg ai/L	Based on test sample results.
Positive control {if used, indicate the chemical and concentrations} :	N/A	
<u>Fertilization success study, if any</u> Number of eggs used:  On what day the eggs were removed to check the embryonic development:	N/A	
Other parameters, if any	Biomass loading at the end of the test was 0.027 g fish/L/day. Instantaneous loading was 0.27 g fish/L at any given time.	Fulfills OCSPP criteria.

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

**2. Observations:**

**Table 2: Observations**

Parameters	Details	Remarks
		Criteria
Parameters measured including the sub-lethal effects/toxicity symptoms:	<ul style="list-style-type: none"> <li>- Time to hatch</li> <li>- Hatching success</li> <li>- Larval survival</li> <li>- Measurement of growth (length, wet and dry weights)</li> <li>- Behavioral and morphological observations</li> </ul>	<p><i>Recommended parameters measured include:</i></p> <ul style="list-style-type: none"> <li>- Number of embryos hatched;</li> <li>- Time to hatch;</li> <li>- Mortality of embryos, larvae, and Juveniles:</li> <li>- Time to swim-up (if appropriate);</li> <li>- Measurement of growth;</li> <li>- Incidence of pathological or Histological effects;</li> <li>- Observations of other effects or clinical signs.</li> </ul>
Observation intervals/dates for:  Egg mortality: No. of eggs hatched: Mortality of fry (e.g.,alevins): Swim-up behavior: Growth measurements: Embryonic development: Other sub-lethal effects	Daily Daily Daily Daily Day 34 Not determined Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	
Other observations, if any	N/A	

**II. RESULTS AND DISCUSSION**

**A. MORTALITY:**

Mean hatching success was 93 to 98% for all control and treatment levels, with no statistically-significant differences indicated. Similarly, post-hatch survival ranged from 93 to 100% for all levels. Thus, the NOAEC and LOAEC for both survival endpoints were 11 and >11 mg ai/L, respectively, based upon mean-measured concentrations.

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

**Table 3: Effect of BAS 183 H (Dicamba acid) on egg hatching and survival at different life stage of fish.<sup>(a)</sup>**

Treatment (mg ai/L) Mean-measured (nominal) concentrations	Egg hatched/embryo viability			Time to hatch, Cumulative No. hatched <sup>(b)</sup>			Percent survival on Day 34
	No. of eggs at study initiation	Hatch/embryo viability		Day 4	Day 5	Day 7	
		No.	%				
Negative Control	80	76	95	1	12	76	93
Solvent Control	80	77	96	1	7	77	99
Pooled Controls	160	153	96	--	--	--	96
0.28 (0.31)	80	77	96	1	1	76	100
0.72 (0.77)	80	77	96	0	7	76	100
1.8 (1.9)	80	78	98	0	1	77	97
4.5 (4.8)	80	74	93	0	1	64	95
11 (12)	80	78	98	0	5	75	97
NOAEC, mg ai/L		11 mg ai/L		11 mg ai/L			11 mg ai/L
LOAEC, mg ai/L		>11 mg ai/L		>11 mg ai/L			>11 mg ai/L
Positive control	N/A						

<sup>(a)</sup> Data were obtained from Table 5 on page 29 of the study report.

<sup>(b)</sup> Reviewer-summed from Appendix 9 on pages 53 & 54 of the study report.

**B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:**

Time to hatch: No treatment-related effect on the time to hatch was observed. Embryos began hatching on Days 4 and 5 of the test and all surviving embryos from all levels had hatched by Day 10, with the majority of embryos hatching on Day 6. The NOAEC and LOAEC for time to hatch were 11 and >11 mg ai/L, respectively, based upon mean-measured concentrations.

Clinical signs of toxicity: In general, the majority of fish from the control and all treatment levels appeared normal throughout the test, with occasional observations of small fish. However, these observations were observed in all groups. The NOAEC and LOAEC for clinical signs of toxicity were 11 and >11 mg ai/L, respectively, based upon mean-measured concentrations.

Growth: Total lengths, wet weights, and dry weights were determined for all surviving fish at study termination. For the negative control, solvent control, and mean-measured 0.28, 0.72, .8, 4.5, and 11 mg ai/L treatment levels, total lengths averaged 19.7, 19.6, 19.1, 19.3, 19.5, 18.7, and 19.3 mm, respectively; wet weights averaged 95.7, 95.6, 86.9, 92.1, 95.6, 86.5, and 97.1 mg, respectively; and dry weights averaged 22.3,

# Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow (*Cyprinodon variegatus*), Early Life Cycle

PMRA Submission Number {.....}

EPA MRID Number 48718011

21.9, 19.9, 21.3, 21.4, 19.5, and 22.1 mg, respectively. Compared to the pooled control, differences were statistically-significant ( $p \leq 0.05$ ) at the 0.28, 0.72, 4.5, and 11 mg ai/L levels for total length, and at the 0.28 and 4.5 mg ai/L levels for wet and dry weights. In all instances, the differences were slight ( $<10\%$ ), did not follow a dose-response pattern, and were not considered by the study author to be a result of exposure. The NOAEC and LOAEC values for all growth endpoints were 11 and  $>11$  mg ai/L, respectively, based upon mean-measured concentrations.

**Table 4: Effect of BAS 183 H (Dicamba acid) on growth of juvenile fish.<sup>(a)</sup>**

Treatment (mg ai/L) Mean-measured (nominal) concentrations	Growth – total length (mm $\pm$ SD)	Growth - wet weight (mg $\pm$ SD)	Growth - dry weight (mg $\pm$ SD)
Negative Control	19.7 $\pm$ 0.22	95.7 $\pm$ 5.4	22.3 $\pm$ 1.1
Solvent Control	19.6 $\pm$ 0.096	95.6 $\pm$ 1.5	21.9 $\pm$ 0.34
Pooled Controls	19.7 $\pm$ 0.18	95.6 $\pm$ 3.7	22.1 $\pm$ 0.77
0.28 (0.31)	19.1 $\pm$ 0.26*	86.9 $\pm$ 2.6*	19.9 $\pm$ 0.83*
0.72 (0.77)	19.3 $\pm$ 0.14*	92.1 $\pm$ 3.9	21.3 $\pm$ 0.85
1.8 (1.9)	19.5 $\pm$ 0.13	95.6 $\pm$ 4.1	21.4 $\pm$ 0.80
4.5 (4.8)	18.7 $\pm$ 0.24*	86.5 $\pm$ 5.4*	19.5 $\pm$ 1.2*
11 (12)	19.3 $\pm$ 0.22*	97.1 $\pm$ 5.2	22.1 $\pm$ 0.86
NOAEC, mg ai/L	11	11	11
LOAEC, mg ai/L	$>11$	$>11$	$>11$
Positive control	N/A		

<sup>(a)</sup> Data were obtained from Table 5 on page 29 of the study report.

\* Significantly different from pooled controls at  $p \leq 0.05$

## C. REPORTED STATISTICS:

**Statistical Method:** Data that were statistically analyzed included hatching success, post-hatch larval survival, and the mean total length, wet weight, and dry weight of surviving fish at study termination. The time to hatch was visually evaluated.

Data obtained for each control group were statistically-compared using a t-test. No statistical differences ( $p \leq 0.05$ ) were observed between the two control groups for any parameter, and thus control data were pooled for subsequent comparisons.

Hatching success and larval survival data (discrete-variable data) were analyzed using Chi-square and Fisher's Exact test to identify treatment groups that showed a statistically significant difference from the pooled control ( $p < 0.05$ ). Growth data (continuous-variable data) were checked for normality using Shapiro-Wilk's test and for

## Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow (*Cyprinodon variegatus*), Early Life Cycle

PMRA Submission Number {.....}

EPA MRID Number 48718011

homogeneity of variance using Levene's test ( $p < 0.01$ ). The data passed these assumptions, and were subsequently analyzed using analysis of variance (ANOVA) followed by Dunnett's test to identify treatments that were significantly different from the pooled control ( $p < 0.05$ ).

The LOAEC, NOAEC, and MATC were reported based upon significance of the data; however, scientific judgment was used to determine if statistical differences were biologically meaningful. All statistical tests were performed using mean-measured concentrations and SAS statistical software.

NOAEC: 11 mg ai/L

LOAEC: >11 mg ai/L

Endpoint(s) affected: none

### D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method(s): The reviewer statistically analyzed the endpoints for hatching success, larval survival, length, wet weight, and dry weight using Toxstat 3.5 statistical software. All analyses were performed using replicate data and mean measured concentrations. The means of the negative and solvent controls were statistically compared for each parameter using t-tests. There were no significant differences between the two control groups and all further analyses were performed by comparing the treatment groups to the negative control only. The data were confirmed to be normally distributed and have homogeneous variances using Shapiro-Wilk's and Levene's tests, respectively, and were therefore analyzed using ANOVA followed by Dunnett's tests. Significant reductions relative to the negative control ( $p < 0.05$ ) were detected for length at the 0.28, 0.72, 4.5, and 11 mg ai/L levels; these inhibitions were consistent, ranging from 2-5% of negative control lengths over the concentration range which was approximately one magnitude order. Significant reductions relative to the negative control were also detected for wet and dry weight at the 0.28 and 4.5 mg ai/L levels; the magnitude of these reductions was greater (i.e., 9-13% lower than negative control weights) than that for length. The time to hatch and clinical signs of toxicity data were visually assessed.

### E. STUDY DEFICIENCIES:

There were no deviations and/or deficiencies from OCSPP guidance affecting the scientific soundness or acceptability of this study.

### F. REVIEWER'S COMMENTS:

The reviewer's statistical conclusions were **not** in agreement with those of the study author. Significant reductions relative to the negative control ( $p < 0.05$ ) were detected for length at the 0.28, 0.72, 4.5, and 11 mg ai/L levels; these inhibitions were consistent, ranging from 2-5% of negative control lengths over the concentration range which was approximately one magnitude order. Significant reductions relative to the negative control were also detected for wet and dry weight at the 0.28 and 4.5 mg ai/L levels; the magnitude of these reductions was greater (i.e., 9-13% lower than negative control weights) than that for length. The dose response for these endpoints, especially for length, was particularly flat over an order of magnitude in dosing. While slight reductions from negative control were observed, they do not appear to be dose-dependent. Based on this information, the reviewer's conclusions are the same as the study authors. The results were provided in terms of mean-measured concentrations. The reviewer calculated % inhibition values for the growth parameters for each treatment level relative to the negative control (see Appendix II).

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

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All validity requirements were met. Specifically, the dissolved oxygen content was maintained at  $\geq 60\%$  throughout the study; the water temperature did not differ by more than  $\pm 1.5^{\circ}\text{C}$  between successive days at any time during the study and was maintained within the temperature range specified for this species; hatching success in the control was  $\geq 75\%$  and post-hatch survival in the controls was  $\geq 80\%$ ; no notable solvent-related effects were detected during the study; and concentrations were satisfactorily maintained within  $\pm 20\%$  of mean-measured concentrations (all levels).

The in-life phase of the definitive study was conducted from September 28 to November 1, 2010.

**G. CONCLUSIONS:**

This study is scientifically sound and thus acceptable. There were no treatment-related effects on time to hatch, hatching success, or post-hatch larval survival. While growth (length, wet weight, and dry weight) was significantly reduced ( $p < 0.05$ ; 3-11% lower than negative control values) at the 0.28 mg ai/L treatment level and reductions from negative control at higher test levels were evident, they were not dose-dependent.

NOAEC: 11mg ai/L

LOAEC: > 11 mg ai/L

Endpoint(s) affected: none

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

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**III. REFERENCES:**

U.S. Environmental Protection Agency. 1996. Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1400: *Fish Early Life-Stage Toxicity Test*.

American Society for Testing and Materials. 2005. ASTM Standard E1241-05. *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fish*.

The SAS System for Windows. 2001. Version 8.2. SAS Institute Inc., Cary, North Carolina.

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

**APPENDIX I: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

Hatching success

Title: Dicamba sheepshead ELS hatching success

File: 8011hs Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 0.9500 Calculated t value = -0.3974

GRP2 (Solvent cntl) Mean = 0.9625 Degrees of freedom = 6

Difference in means = -0.0125

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05

2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba sheepshead ELS hatching success

File: 8011hs Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.0238

W = 0.9478

Critical W = 0.8840 (alpha = 0.01 , N = 24)

W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba sheepshead ELS hatching success

File: 8011hs Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0012	0.0002	0.5143
Within (Error)	18	0.0088	0.0005	
Total	23	0.0100		

(p-value = 0.7620)

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

Critical F = 4.2479 (alpha = 0.01, df = 5,18)  
= 2.7729 (alpha = 0.05, df = 5,18)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All equal (alpha = 0.01)

Title: Dicamba sheepshead ELS hatching success  
File: 8011hs Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0025	0.0005	0.3789
Within (Error)	18	0.0237	0.0013	
Total	23	0.0262		

(p-value = 0.8566)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)  
= 2.7729 (alpha = 0.05, df = 5,18)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All equal (alpha = 0.05)

Title: Dicamba sheepshead ELS hatching success  
File: 8011hs Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2  $H_0$ :Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
0.05					
1	neg control	0.9500	0.9500		
2	0.28	0.9625	0.9625	-0.4867	
3	0.72	0.9625	0.9625	-0.4867	
4	1.8	0.9750	0.9750	-0.9733	
5	4.5	0.9500	0.9500	0.0000	
6	11	0.9750	0.9750	-0.9733	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba sheepshead ELS hatching success  
File: 8011hs Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2  $H_0$ :Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

2	0.28	4	0.0619	6.5	-0.0125
3	0.72	4	0.0619	6.5	-0.0125
4	1.8	4	0.0619	6.5	-0.0250
5	4.5	4	0.0619	6.5	0.0000
6	11	4	0.0619	6.5	-0.0250

Larval survival

Title: Dicamba sheepshead ELS larval survival (end of test)

File: 8011ls Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 0.9325 Calculated t value = -1.5378

GRP2 (Solvent cntl) Mean = 0.9875 Degrees of freedom = 6

Difference in means = -0.0550

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05

2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba sheepshead ELS larval survival (end of test)

File: 8011ls Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.0235

W = 0.9177

Critical W = 0.8840 (alpha = 0.01 , N = 24)

W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba sheepshead ELS larval survival (end of test)

File: 8011ls Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0057	0.0011	2.2726
Within (Error)	18	0.0089	0.0005	

# Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow

(*Cyprinodon variegatus*), Early Life Cycle

PMRA Submission Number {.....}

EPA MRID Number 48718011

Total	23	0.0146
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(p-value = 0.0910)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)  
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.01)

Title: Dicamba sheepshead ELS larval survival (end of test)

File: 80111s

Transform:

NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	0.0150	0.0030	2.2904
Within (Error)	18	0.0235	0.0013	
Total	23	0.0385		

(p-value = 0.0891)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)  
= 2.7729 (alpha = 0.05, df = 5,18)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Title: Dicamba sheepshead ELS larval survival (end of test)

File: 80111s

Transform:

NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
0.05					
1	neg control	0.9325	0.9325		
2	0.28	1.0000	1.0000	-2.6391	
3	0.72	1.0000	1.0000	-2.6391	
4	1.8	0.9750	0.9750	-1.6617	
5	4.5	0.9475	0.9475	-0.5865	
6	11	0.9750	0.9750	-1.6617	

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba sheepshead ELS larval survival (end of test)

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

File: 80111s Transform: NO TRANSFORMATION

Dunnett's Test		-	TABLE 2 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION		NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control		4			
2	0.28		4	0.0616	6.6	-0.0675
3	0.72		4	0.0616	6.6	-0.0675
4	1.8		4	0.0616	6.6	-0.0425
5	4.5		4	0.0616	6.6	-0.0150
6	11		4	0.0616	6.6	-0.0425

Length

Title: Dicamba sheepshead ELS length (mm)

File: 80111 Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 19.7250 Calculated t value = 1.2421

GRP2 (Solvent cntl) Mean = 19.5750 Degrees of freedom = 6

Difference in means = 0.1500

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05

2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba sheepshead ELS length (mm)

File: 80111 Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 0.7900

W = 0.9463

Critical W = 0.8840 (alpha = 0.01 , N = 24)

W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba sheepshead ELS length (mm)

File: 80111 Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
--------	----	----	----	---

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

Between	5	0.0600	0.0120	1.0286
Within (Error)	18	0.2100	0.0117	
Total	23	0.2700		

(p-value = 0.4305)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)  
= 2.7729 (alpha = 0.05, df = 5,18)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All equal (alpha = 0.01)

Title: Dicamba sheepshead ELS length (mm)

File: 80111

Transform:

NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	2.4483	0.4897	11.1570
Within (Error)	18	0.7900	0.0439	
Total	23	3.2383		

(p-value = 0.0001)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)  
= 2.7729 (alpha = 0.05, df = 5,18)

Since  $F > \text{Critical } F$  REJECT  $H_0$ : All equal (alpha = 0.05)

Title: Dicamba sheepshead ELS length (mm)

File: 80111

Transform:

NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2

$H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
0.05					
1	neg control	19.7250	19.7250		
2	0.28	19.0750	19.0750	4.3878	*
3	0.72	19.3000	19.3000	2.8690	*
4	1.8	19.4750	19.4750	1.6876	
5	4.5	18.7000	18.7000	6.9193	*
6	11	19.2750	19.2750	3.0377	*

Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

Title: Dicamba sheepshead ELS length (mm)  
File: 80111 Transform: NO TRANSFORMATION

Dunnett's Test		- TABLE 2 OF 2		Ho:Control<Treatment		
GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL	
1	neg control	4				
2	0.28	4	0.3570	1.8	0.6500	
3	0.72	4	0.3570	1.8	0.4250	
4	1.8	4	0.3570	1.8	0.2500	
5	4.5	4	0.3570	1.8	1.0250	
6	11	4	0.3570	1.8	0.4500	

Wet weight

Title: Dicamba sheepshead ELS wet weight (mg)  
File: 8011ww Transform: NO TRANSFORMATION

t-Test of Solvent and Blank Controls Ho: GRP1 Mean = GRP2 Mean

GRP1 (Blank cntl) Mean = 95.6750 Calculated t value = 0.0443  
GRP2 (Solvent cntl) Mean = 95.5500 Degrees of freedom = 6  
Difference in means = 0.1250

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05  
2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba sheepshead ELS wet weight (mg)  
File: 8011ww Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 372.4600  
W = 0.9591

Critical W = 0.8840 (alpha = 0.01 , N = 24)  
W = 0.9160 (alpha = 0.05 , N = 24)

Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba sheepshead ELS wet weight (mg)  
Page 23 of 29

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

File: 8011ww Transform: NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	24.1733	4.8347	0.7468
Within (Error)	18	116.5300	6.4739	
Total	23	140.7033		

(p-value = 0.5989)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)  
= 2.7729 (alpha = 0.05, df = 5,18)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All equal (alpha = 0.01)

Title: Dicamba sheepshead ELS wet weight (mg)

File: 8011ww Transform: NO TRANSFORMATION

ANOVA Table

SOURCE	DF	SS	MS	F
Between	5	431.1200	86.2240	4.1670
Within (Error)	18	372.4600	20.6922	
Total	23	803.5800		

(p-value = 0.0109)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)  
= 2.7729 (alpha = 0.05, df = 5,18)

Since  $F > \text{Critical } F$  REJECT  $H_0$ : All equal (alpha = 0.05)

Title: Dicamba sheepshead ELS wet weight (mg)

File: 8011ww Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 1 OF 2  $H_0$ : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
0.05					
1	neg control	95.6750	95.6750		
2	0.28	86.8750	86.8750	2.7359	*

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

3	0.72	92.1250	92.1250	1.1037
4	1.8	95.5750	95.5750	0.0311
5	4.5	86.5000	86.5000	2.8524 *
6	11	97.0500	97.0500	-0.4275

-----  
Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)

Title: Dicamba sheepshead ELS wet weight (mg)

File: 8011ww

Transform:

NO TRANSFORMATION

Dunnett's Test		-	TABLE 2 OF 2	Ho:Control<Treatment		
GROUP	IDENTIFICATION		NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	neg control		4			
2	0.28		4	7.7519	8.1	8.8000
3	0.72		4	7.7519	8.1	3.5500
4	1.8		4	7.7519	8.1	0.1000
5	4.5		4	7.7519	8.1	9.1750
6	11		4	7.7519	8.1	-1.3750

Dry weight

Title: Dicamba sheepshead ELS dry weight (mg)

File: 8011dw

Transform:

NO TRANSFORMATION

t-Test of Solvent and Blank Controls

Ho: GRP1 Mean = GRP2 Mean

=====

GRP1 (Blank cntl) Mean = 22.3000 Calculated t value = 0.7127

GRP2 (Solvent cntl) Mean = 21.9000 Degrees of freedom = 6

Difference in means = 0.4000

=====

2-sided t value (0.05, 6) = 2.4469 No significant difference at alpha=0.05

2-sided t value (0.01, 6) = 3.7074 No significant difference at alpha=0.01

WARNING: This procedure assumes normality and equal variances!

Title: Dicamba sheepshead ELS dry weight (mg)

File: 8011dw

Transform:

NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

-----

D = 16.1975

W = 0.9477

Critical W = 0.8840 (alpha = 0.01 , N = 24)

W = 0.9160 (alpha = 0.05 , N = 24)

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

-----  
Data PASS normality test (alpha = 0.01). Continue analysis.

Title: Dicamba sheepshead ELS dry weight (mg)

File: 8011dw

Transform:

NO TRANSFORMATION

Levene's Test for Homogeneity of Variance

ANOVA Table

-----

SOURCE	DF	SS	MS	F
Between	5	0.6221	0.1244	0.3850
Within (Error)	18	5.8175	0.3232	
Total	23	6.4396		

-----

(p-value = 0.8526)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)

= 2.7729 (alpha = 0.05, df = 5,18)

Since  $F < \text{Critical } F$  FAIL TO REJECT  $H_0$ : All equal (alpha = 0.01)

Title: Dicamba sheepshead ELS dry weight (mg)

File: 8011dw

Transform:

NO TRANSFORMATION

ANOVA Table

-----

SOURCE	DF	SS	MS	F
Between	5	25.8288	5.1658	5.7406
Within (Error)	18	16.1975	0.8999	
Total	23	42.0263		

-----

(p-value = 0.0024)

Critical F = 4.2479 (alpha = 0.01, df = 5,18)

= 2.7729 (alpha = 0.05, df = 5,18)

Since  $F > \text{Critical } F$  REJECT  $H_0$ : All equal (alpha = 0.05)

Title: Dicamba sheepshead ELS dry weight (mg)

File: 8011dw

Transform:

NO TRANSFORMATION

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

Dunnett's Test		-	TABLE 1 OF 2	Ho:Control<Treatment	
GROUP	IDENTIFICATION		TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT
0.05					SIG
1	neg control		22.3000	22.3000	
2	0.28		19.9250	19.9250	3.5407 *
3	0.72		21.2750	21.2750	1.5281
4	1.8		21.3750	21.3750	1.3790
5	4.5		19.5250	19.5250	4.1370 *
6	11		22.1250	22.1250	0.2609
Dunnett critical value = 2.4100 (1 Tailed, alpha = 0.05, df = 5,18)					

Title: Dicamba sheepshead ELS dry weight (mg)

File: 801ldw

Transform:

NO TRANSFORMATION

Dunnett's Test		-	TABLE 2 OF 2	Ho:Control<Treatment	
GROUP	IDENTIFICATION		NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL
					DIFFERENCE FROM CONTROL
1	neg control		4		
2	0.28		4	1.6166	7.2
3	0.72		4	1.6166	7.2
4	1.8		4	1.6166	7.2
5	4.5		4	1.6166	7.2
6	11		4	1.6166	7.2

# Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow (*Cyprinodon variegatus*), Early Life Cycle

PMRA Submission Number {.....}

EPA MRID Number 48718011

## APPENDIX II. PERCENT INHIBITION OF GROWTH PARAMETERS RELATIVE TO THE NEGATIVE CONTROL CALCULATED IN MICROSOFT EXCEL

Concentration	Mean length	% inhibition	Mean wet weight	% inhibition	Mean dry weight	% inhibition
Control	19.7	0%	95.7	0%	22.3	0%
0.28	19.1	3%	86.9	9%	19.9	11%
0.72	19.3	2%	92.1	4%	21.3	4%
1.8	19.5	1%	95.6	0%	21.4	4%
4.5	18.7	5%	86.5	10%	19.5	13%
11	19.3	2%	97.1	-1%	22.1	1%

**Data Evaluation Report on the Toxicity of Dicamba Acid to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**

PMRA Submission Number {.....}

EPA MRID Number 48718011

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